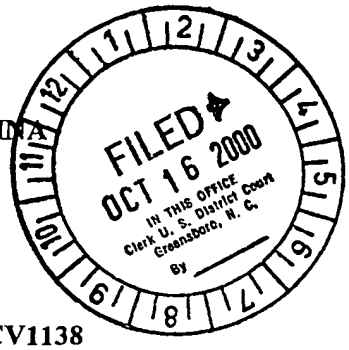
APPENDIX 9

IN THE UNITED STATES DISTRICT COURT
FOR THE MIDDLE DISTRICT OF NORTH CAROLINA



RHÔNE-POULENC AGRO S.A.,	)	
(Now known as Aventis CropScience SA)	)	
	)	
Plaintiff,	)	
y.	)	**C.A. NO. 1:97CV1138**
	)	
MONSANTO COMPANY	)	
(Now known as Pharmacia Corporation)	)	
	)	
and	)	
	)	
DEKALB GENETICS CORPORATION,	)	
	)	
Defendants.	)	
	)	

RPA'S MOTION TO STRIKE

RPA hereby respectfully moves to strike Section IA (pages 1- 9) of DeKalb's Response to RPA's Post-Trial Brief of Findings of Fact and Conclusions of Law on the grounds that it raises extensive new arguments regarding Dr. Luca Comai and Calgene that were required to have been raised in DeKalb's initial Post-Trial Brief, but were not.

I. Factual Background

DeKalb devoted a significant portion of trial to cross-examining RPA's witnesses and introducing its own evidence relation to Dr. Luca Comai and Calgene. This was introduced in order to support DeKalb's contention that RPA did not make any significant contribution to the claims of the patents in suits because RPA's DNA constructs were somehow derived from Calgene.

By Order dated September 20, 2000, this Court established the briefing schedule for the parties' post-trial briefs on proposed findings of fact and conclusions of law. (Docket # 669).

In its Post-Trial Brief of Findings of Fact and Conclusions of Law, filed its on September 29, 2000, RPA addressed what it understood to be DeKalb's contentions relating to Dr. Comai and Calgene. *See* RPA's Post-Trial Brief at 8 - 12, 32 - 35.

In its primary submission filed on September 29, 2000, however, DeKalb virtually ignored its contentions relating to Dr. Comai and Calgene. DeKalb's entire briefing on these issues is found in a single paragraph on page 24 of its submission

On October 6, 2000, the parties filed their replies to each other's submissions. RPA did not address Dr. Comai and Calgene in that reply, because DeKalb did not submit anything requiring a response. DeKalb's purported reply, however, contains nine pages of alleged findings of facts and arguments devoted to its contentions about Dr. Comai's alleged contributions. Many of the arguments are surprising and new, and RPA never had any opportunity to respond to them.

II. ARGUMENT

Section IA of DeKalb's Response to RPA's Post-Trial Brief is inconsistent with the Court's September 20 Order and the Local Rules. The Court should strike Section 1A of DeKalb's brief or, in the alternative, allow RPA to respond to that section of the brief.

The Court clearly ordered that the parties file their complete initial briefs on September 29 that would address all of the key issues, and certainly the issues upon which the party was relying to support or defeat the jury verdict. DeKalb impermissibly withheld its arguments about Dr. Comai and Calgene until its reply brief. RPA did not have a chance to respond under the Court's Order. Yet, DeKalb's new arguments contain many errors.

III. Conclusion

For the reasons set forth above, RPA respectfully requests that the Court strike Section IA of DeKalb's Response to RPA's Post-Trial Brief of Findings of Fact and Conclusions of Law. In the alternative, RPA respectfully requests that the Court allow RPA to file a surreply responding to the new arguments contained in Section 1A of DeKalb's response.

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Date: October 16, 2000

CERTIFICATE OF SERVICE

I hereby certify that on this day of October 16, 2000, a true and correct copy of the attached document was caused to be served on the attorneys of record at the addresses as indicated below by the methods of service indicated below:

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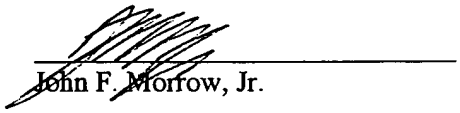

John F. Morrow, Jr.

REPORT

Comparison of Glyphosate Tolerance of Four Coding Sequences in Transgenic Tobacco

Prepared by: Alain Sailland, PhD and Jean-Marc Ferrullo, PhD

SUMMARY

At the request of counsel for Aventis, we have compared the respective tolerance to the herbicide glyphosate in tobacco provided by a common expression cassette with four different coding sequences for expression of: (1) a chloroplast transit peptide ("OTP") fused to double mutant *Zea mays* EPSPS (102/103Thr→Ile, 106/107Pro→Ser) sequence ("DMMG"); (2) OTP fused to single mutant *Salmonella typhimurium* bacterial EPSPS (101Pro→Ser) sequence ("CT7"); (3) OTP fused to double mutant *Salmonella typhimurium* bacterial EPSPS (97Thr→Ile, 101Pro→Ser) sequence ("DMAroA"); and (4) the first two elements of the OTP, i.e., *Helianthus annuus* RuBisCo ssu chloroplast transit peptide fused to the N-terminal 22 amino acids of *Zea mays* RuBisCo ssu ("CTPha+22AA mz"), fused to DMMG. These DNA constructs will be referred to respectively as (1) OTP-DMMG; (2) OTP-CT7; (3) OTP-DMAroA; and (4) CTPha+22AAmz-DMMG.

The results demonstrate that using the tested common expression cassette, OTP-DMMG is significantly superior to OTP-DMAroA in providing glyphosate tolerance to tobacco plants, and CTPha+22AAmz-DMMG and OTP-CT7 provided insubstantial glyphosate tolerance.

METHODOLOGY

DNA construction, manipulation and analyses were all performed according to standard methods (Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K., eds. (1993) in Current Protocols in Molecular Biology (Wiley, New York))

1. **Vector Construction**

Expression Cassette

The same expression cassette was used in all transformations, comprising in the direction of transcription a double histone promoter, coding sequence and a NOS terminator. This cassette has demonstrated best expression of glyphosate tolerant genes in tobacco plants in our laboratory.

Elements

Double histone promoter is derived from H4A748, a clone from Arabidopsis thaliana, Strasbourg strain, described by M.E. Chaboute (thesis from the University of Strasbourg, 1987) and Plant Mol. Biol. Vol 8, 1987 p 179-191; PubMed Accession M17132; see also "A Plant Histone Gene Promoter Can Direct Both Replication-dependent and Independent Gene Expression in Transgenic Plants", Lepetit M, Ehling M, Chaubet N, Gigot C, Mol Gen Genet 1992 Jan;231(2):276-85; "A 126 bp Fragment of a Plant Histone Gene Promoter Confers Preferential Expression in Meristems of Transgenic Arabidopsis", Atanassova R, Chaubet N, Gigot C, Plant J 1992 May;2(3):291-300; U.S. Patents 5,491,288 and 5,792,930. The sequence is (see Exhibit 1):

SacI ~~~~~~ EcoRI PstI ~~~~~~ ~~~~~~

2752 GAATTCGAGCTC GAATTGGCCGCT CTAGAGCTTGCA TGCCTGCAGGTC GAGGAGAAATAT

GAGTCGAGGCAT

2824 GGATACACTAAG TTCCCCTGAAGT GAGCATGATCTT TGATGCTGAGAT GATTCCCAGAGC

AAGATAGTTTGT

2896 GCTGCAAGTGAC ACAATTGTAATG AAACCACCACTC AACGAATTTACT TGTGGCTTTGAC

ATGTCGTGTGCT

2968 CTGTTTGTATTT GTGAGTGCCGGT TGGTAATTATTT TTGTTAATGTGA TTTTAAAACCTC

TTATGTAAATAG

3040 TTACTTTATCTA TTGAAGTGTGTT CTTGTGGTCTAT AGTTTCTCAAAG GGAAATTAAAAT

GTTGACATCCCA

3112 TTTACAATTGAT AACTTGGTATAC ACAAACTTTGTA AATTTGGTGATA TTTATGGTCGAA

AGAAGGCAATAC

3184 CCATTGTATGTT CCAATATCAATA TCAATACGATAA CTTGATAATACT AACATATGATTG

TCATTGTTTTTC

3256 CAGTATCAATAT ACATTAAGCTAC TACAAAATTAGT ATAAATCACTAT ATTATAAATCTT

TTTCGGTTGTAA

3328 CTTGTAATTCGT GGGTTTTTAAAA TAAAAGCATGTG AAAATTTTCAAA TAATGTGATGGC

GCAATTTTATTT

3400 TCCGAGTTCCAA AATATTGCCGCT TCATTACCCTAA TTTGTGGCGCCA CATGTAAAACAA

AAGACGATTCTT

3472 AGTGGCTATCAC TGCCATCACGCG GATCACTAATAT GAACCGTCGATT AAAACAGATCGA

CGGTTTATACAT

3544 CATTTTATTGTA CACACGGATCGT ATGATTGTCATT GTTTTTCCAGTA TCAATATACATT

AAGCTACTACAA

3616 AATTAGTATAAA TCACTATATTAT AAATCTTTTTCG GTTGTAACTTGT AATTCGTGGGTT

TTTAAAATAAAA

3688 GCATGTGAAAAT TTTCAAATAATG TGATGGCGCAAT TTTATTTTCCGA GTTCCAAAATAT

TGCCGCTTCATT

3760 ACCCTAATTTGT GGCGCCACATGT AAAACAAAAGAC GATTCTTAGTGG CTATCACTGCCA

TCACGCGGATCA

ClaI ~~~~

3832 CTAATATGAACC GTCGATTAAAAC AGATCGACGGTT TATACATCATTT TATTGTACACAC

GGATCGATATCT

3904 CAGCCGTTAGAT TTAATATGCGAT CTGATTGCTCAA AAAATAGACTCT CCGTCTTTGCCT

ATAAAAACAATT

3976 TCACATCTTTCT CACCCAAATCTA CTCTTAACCGTT CTTCTTCTTCTA CAGACATCAATT

TCTCTCGACTCT

PstI ~~~~~~ EcoRI NcoI ~~~~~ ~~~~

4048 AGAATTCCTGCA GCCCCATGG

OTP is the optimized transit peptide described in United States Patent RE36,449, a nucleic acid construct which codes for a polypeptide which in the direction of translation comprises a first chloroplast transit peptide from *Helianthus annuus* ribulose-1,5-bisphosphate carboxylase small subunit (Waksman, G et al "Nucleotide Sequence of a Gene Encoding Sunflower Ribulose-1,5-bisphosphate Carboxylase/oxygenase Small Subunit (Rbcs)", Nucleic Acids Research, vol. 15, No. 17:7181 (1987); PubMed Accession Y00431), 22 amino acids from the N-terminal region of a mature *Zea mays* ribulose-1,5-bisphosphate carboxylate small subunit (LeBrun M et al "Nucleotide Sequence of a Gene Encoding Corn Ribulose-1,5 Bisphosphate Carboxylase/Oxygenase Small Subunit (rbcs)", Nucleic Acids Research, vol. 15, No. 10: 4360 (1987); PubMed Accession Y00322), and a second chloroplast transmit peptide from a *Zea mays* ribulose-1,5-bisphosphate carboxylate small subunit (*Id.*), modified by PCR by DeRose, R. (TPha TGC→TGT, and mature zm ssu TCG→TCT; unpublished) (Seq. 4065 - 4437 of Exhibit 1).

CTPha+22AAmz is a nucleic acid construct which codes for a polypeptide which in the direction of translation comprises a first chloroplast transit peptide from *Helianthus annuus* ribulose-1,5-bisphosphate carboxylase small subunit (Waksman, G et al "Nucleotide Sequence of a Gene Encoding Sunflower Ribulose-1,5-bisphosphate Carboxylase/oxygenase Small Subunit (Rbcs)", Nucleic Acids Research, vol. 15, No. 17:7181 (1987); PubMed Accession Y00431), and 22 amino acids from the N-terminal region of a mature *Zea mays* ribulose-1,5-bisphosphate carboxylate small subunit (LeBrun M et al "Nucleotide Sequence of a Gene Encoding Corn Ribulose-1,5 Bisphosphate Carboxylase/Oxygenase Small Subunit (rbcs)", Nucleic Acids Research, vol. 15, No. 10: 4360 (1987); PubMed Accession Y00322),

DMMG is the *Zea mays* 5-enolpyruvylshikimate-3-phosphate synthase ("EPSPS") nucleic acid sequence described in PubMed Accession CAA44974, modified to add ATG start and mutated at nucleotides 304-306 (ACT→ATC) and 316-318 (CCA→TCC) (Seq. 4438 - 5772 of Exhibit 1).

DMAroA is the *Salmonella typhimurium* EPSPS nucleic acid sequence, mutated at nucleotides 315-317 (ACC→ATC) and 327-329 (CCG→TCG) (Seq. 5735 - 992 of Exhibit 4).

The NOS terminator is conventional, having sequence:

5795 GAATTTCCCCGA TCGTTCAAACAT TTGGCAATAAAG TTTCTTAAGATT GAATCCTGTTGC

CGGTCTTGCGAT

5867 GATTATCATATA ATTTCTGTTGAA TTACGTTAAGCA TGTAATAATTAA CATGTAATGCAT

GACGTTATTTAT

5939 GAGATGGGTTTT TATGATTAGAGT CCCGCAATTATA CATTTAATACGC GATAGAAAACAA

AATATAGCGCGC

KpnI ~ EagI ~~~~~~

6011 AAACTAGGATAA ATTATCGCGCGC GGTGTCATCTAT GTTACTAGATCG GGAATTGCGGCC GGGTACC

preceded by a spacer: between DDMG and NOS: GCTCTAGAAGAAGCTTCGAC;

and between DMaroA and NOS: GTCTTCTGTTGCGCCAGTCGAC

OTP/DMMG

The plasmid pRD2010-sac, available at the laboratory, contains the expression cassette 2xH4A748 - OTP - DMMG - NOS in a pUC-based vector (Exhibit 1).

CTPha+22AAmz/DMMG

The NruI-BglII fragment from pRD2010-sac containing the entire OTP sequence was excised and replaced by the NruI-BglII fragment from pRD124, a vector available at the laboratory, in order to substitute OTP with the CTPha+22AAmz portion of the OTP. The plasmid was made November 24,1999 and was designated PEPS2 (Exhibit 2).

OTP-CT7

The Nco-EagI fragment from pRD2010-sac containing OTP-DMMG-NOS was excised and replaced by the Nco-EagI fragment from pRD14, a vector available at the laboratory containing an OTP-CT7-NOS cassette, to give pCH90 (obtained September 20, 1999) (Exhibit 3).

OTP-DMAroA

Site-directed-mutagenesis was performed on pCH90 using U.S.E. mutagenesis kit (Pharmacia) with the oligonucleotide 5'(phosphate)-cggtaatgccggaatcgcgatgcgttcgttagcg-3' to give pCH91, in which the single mutant CT7 was changed into a double mutant aroA (97Thr→Ile + 101Pro→Ser). PCH 91 was obtained in the laboratory on September 27, 1999 (Exhibit 4).

2. Plant Transformation and Regeneration

The SacI-KpnI fragments of pRD2010-sac, pEPS2, pCH90, pCH91 were subcloned in T-DNA of apBin19-based binary vector to provide transformation vectors pEPS0 Bin, pEPS2 Bin, pCH90 Bin, and pCH91 Bin. pEPS2 Bin was obtained on November 30, 1999; pCH90 Bin and pCH91 Bin were obtained on December 14, 1999:

pEPS0: OTP-DMMG

pEPS2: CTPha+22AAmz-DMMG

pCH90: OTP-CT7

pCH91: OTP-DMAroA

Tobacco was transformed using standard techniques. The four vector plasmids were transformed into *Agrobacterium tumefaciens* strain EHA105 using the eletroporation method. Plant material was Nicotiana tabacum cv. Petit Havana SR1 (Maliga, P., Sz.-Breznotivits, A., Marton, L. & Joo, F. (1975) Nature (London) 255, 401-402). The leaves were sterilized with 1% sodium hypochlorite and cut into leaf disks of approximately 0.8 cm. These leaf segments were inoculated for 10 min with an overnight culture of diluted bacteria and put on a sterilized filter paper to remove the bacteria in suspension. The infected leaf segments were co-cultivated for 3 days. Plants were selected on plates using Kanamycin (100 mg/l) as a selective agent. Plantlets were regenerated in accordance with standard methods, and allowed to produce seed..

pEPS0: Ten (10) transgenic tobacco plantlets transformed with pEPS0 Bin were transferred into greenhouse (T0 transformants were identified as pEPS0-1 to pEPS0-10) and T1 seeds were harvested.

pEPS2: Twenty one (21) transgenic tobacco plantlets transformed with pEPS2 Bin were transferred

into greenhouse (T0 transformants were identified as pEPS2-1 to pEPS2-21) and T1 seeds were harvested.

pCH90: Nine (9) transgenic tobacco plantlets transformed with pCH90 Bin were transferred into greenhouse (T0 transformants were identified as pCH90-1 to pCH90-9) and T1 seeds were harvested.

pCH91: Eight (8) transgenic tobacco plantlets transformed with pCH91 Bin were transferred into greenhouse (T0 transformants were identified as pCH91-1 to pCH91-8) and T1 seeds were harvested.

3. Trials for Glyphosate Tolerance

Ten (10) T1 seeds from each transformant event were placed in soil on February 7, 2001, and grown in the greenhouse from seed to 4-leaf stage (approx. 4cm height). On March 7, 2001, all plants were sprayed with a commercial Roundup® formulation (360g/L a.i.[acid]) at a dose equivalent to 4 kg/ha of glyphosate acid. Spraying was done in a spray hood (ARO AG Machinenbau ingenieurbüüro 49010 Langenthal). Plants are disposed as a single column under the nozzle, with enough space between pots so that no plant parts are shaded by another plant. The time-course of the nozzle from the left to the right is fixed. The system was calibrated before each treatment by placing a square of filter paper of known surface under the flow (at a height corresponding to the height of top leafs of the material to be treated) and then weighing the filter paper. The gain of mass of the paper provides the dose per hectare.

The tolerance of plants to the treatment with glyphosate was evaluated at 9 days after treatment and 18 days after treatment.

Field trials of the same events will be attempted this summer.

4. Toxicity

Prior testing by Calgene and this laboratory have demonstrated that the bacterial aroA genes

containing the Thr-Ile mutation at position 97 become toxic in bacteria. A roughly equivalent mutation in the DMMG does not induce toxicity. The DMMG and DMAroA materials in these tests are being tested to determine if the same toxicity pattern remains true.

RESULTS

The results are reported in the tables 1 and 2 below. Phytotoxicity is defined as stunting and/or yellowing.

Table 1: Evaluation 9 days after treatment at 4 kg/ha (glyphosate acid)

9 days	Tolerant, no phyto		Tolerant, phyto		Dead	
PEPS0	18/100	18%	5/100	5%	77/100	77%
PEPS2	27/210	12.86%	17/210	8.1%	166/210	79.05%
PCH90	0/90	0%	24/90	26.7%	66/90	73.3%
PCH91	7/80	8.75%	20/80	25%	53/80	66.25%

Table 2: Evaluation 18 days after treatment at 4 kg/ha (glyphosate acid)

18 days	Tolerant, no phyto		Tolerant, phyto		Dead	
PEPS0	17/100	17%	6/100	6%	77/100	77%
PEPS2	0/210	0%	44/210	20.95%	166/210	79.05%
PCH90	0/90	0%	0/90	0%	90/90	100%
PCH91	0	0	24/80	30%	56/80	70%

In cases where some level of tolerance has been shown, the general ratio of dead plants to those showing at least some level of tolerance is consistent with historical results and is conventional.

Pictures of all surviving plants were taken at 18 days after treatment are shown in exhibits 5 to 10. No pictures were taken of pCH90 plants (all dead).

Exhibit 5: pEPS0 (OTP-DMMG)

Exhibit 6: pEPS2 (CTPha+22AAmz-DMMG)

Exhibit 7: pCH91 (OTP-DMAroA)

Exhibit 8: comparison pEPS0 (left) and pEPS2 (right) (OTP-DMMG and CTPha+22AAmz-DMMG)

Exhibit 9: comparison pEPS0 (left) and pCH91 (right) (OTP-DMMG and OTP-DMAroA)

Exhibit 10: comparison of best plants from pEPS0 (left), pCH91 (center) and pEPS2 (right) (OTP-DMMG; OTP-DMAroA; and CTPha+22AAmz-DMMG)